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Presented are five procedures which permit the effective teaching of chromatography with equipment which is readily available, economical, and simple in design. The first procedure involves a study of solute partition in two immiscible solvents and of countercurrent distribution. The second illustrates the use of unidimensional ascending paper chromatography in separating the same mixture of colored components used in the first procedure. The third procedure illustrates the separation of three common sugars by unidimensional ascending paper chromatography. The fourth procedure demonstrates the separation of amino acids by ascending two-dimensional paper chromatography. The fifth procedure involves the separation of a dye mixture using thin-layer chromatography. (DS)

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USE CHROMATOGRAPHY

as a science teaching aid

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WHY TEACH CHROMATOGRAPHY?

Chromatography is an extremely efficient technique for the separation of mixtures of chemical substances. It was named and popularized as a practical method of chemical analysis by Mikhail Tswett, a Russian botanist who, in 1903, described the successful separation of a mixture of colored plant pigments using a simple glass column filled with calcium carbonate.[1] Since that time, the procedure has been modified, improved, and extended so that today practically all disciplines of chemistry utilize some form of chromatography for both colored and non-colored materials.

This technique is widely used for the microanalysis of fission products of atomic piles, of foods for the detection of additives, and of drugs, dyes, pesticides, perfumes, vitamins, antibiotics, and hormones. In medicine it plays a most important role in the detection of normal and abnormal constituents of blood, urine, saliva, and other body tissues. It has helped scientists to find metabolic pathways and has helped to clarify various genetic defects.

In view of its general applicability and versatility, it would be desirable to include in science programs some training in the essentials of chromatography. One could seriously question the practicality of a teaching program in chromatography, however, if he considers the cost of commercially available chromatography equipment, the space required for such equipment, and the time available for student participation in chromatography exercises. These realistic limitations were considered in the design of this series of five procedures in chromatography which permit the effective teaching of the subject with equipment which is readily available, economical, and simple in design and use. All of the procedures may be carried out during ordinary classroom or laboratory periods by the students themselves or as demonstrations by the teachers.

Instruction of the subject matter follows a logical sequence, with each procedure stressing a particular aspect of chromatography. The interrelationships become apparent to the student as he progresses from the simple to the comparatively more complex techniques. More sophisticated chromatographic procedures [1] such as column chromatography, electrochromatography, ion exchange, and gas chromatography become more understandable and meaningful to the student upon completion of these basic procedures.

The first procedure, in two parts, involves a study of solute partition in two immiscible solvents and of countercurrent distribution. It employs a separatory funnel or test tube, to effect the separation of a mixture of colored components. A second procedure illustrates the use of unidimensional ascending paper chromatography for the separation of the same mixture. The latter method employs an ordinary test tube and cork for the chromatography "chamber." A third procedure illustrates the separation of three common sugars by unidimensional ascending paper chromatography. The fourth procedure demonstrates the separation of amino acids, accomplished by ascending two-dimensional paper chromatography, and a fifth involves the separation of a dye mixture using thin-layer chromatography.

PROCEDURE 1: ILLUSTRATION OF SOLVENT PARTITION OF SOLUTES

The student must first understand the concept of solute separation in a two-phase solvent system to appreciate the effectiveness of chromatographic techniques. This can best be demonstrated by using a separatory funnel, a mixture of solutes, and two immiscible solvents in which the solutes are soluble to a differential degree. The degree of solubility of each component is based upon its chemical nature

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or affinity for a particular solvent phase. If two solutes, A and B, with widely differing solubility characteristics are used, such that one solute is highly soluble in one solvent phase and the other is highly soluble in the other phase, the solutes will become quickly distributed, or partitioned, into the appropriate solvent phase. In chromatography, such distributions are expressed by the term *partition coefficient* and designated by the symbol a which is defined as the ratio of the solubilities of each solute in the respective solvent phases at equilibrium. Mathematically,

$$a_A = \frac{\text{concentration of solute A in upper (less dense) phase}}{\text{concentration of solute A in lower (heavier) phase}}$$

$$a_B = \frac{\text{concentration of solute B in upper solvent phase}}{\text{concentration of solute B in lower solvent phase}}$$

A consideration of a will indicate the degree of solubility or separation of the solutes. For example, if the solutes have a values of 0.2 and 0.3 in a given two-phase solvent system, this would indicate a similarity in solubility of each solute in each of the two immiscible solvent phases. These solutes would be difficult to separate by solvent partition. If, however, the a values were 0.2 and 8.0, the solutes would be differentially soluble in each phase and consequently easily separable.

Part A: Solute Partition by Separatory Funnel

If a solute mixture of a dye, sudan IV, and two indicators, bromcresol green and congo red, are added to a separatory funnel containing the immiscible two-phase solvent system chloroform and water, the solutes become partitioned according to their solubility in the respective solvents after mixing and settling of the solvents. Sudan IV, being more chloroform-soluble, will predominate in the chloroform layer, and in contrast, the congo red and bromcresol green will remain in the water phase. If the lower chloroform phase is removed and additional chloroform extractions of the water phase are made, additional chloroform-soluble dye will be extracted each time, but in diminishing quantities.

Materials[1]
Separatory funnel[2]
3 test tubes to collect extracts
Chloroform
Indicator dye mixture[3]

Add 200 milligrams (mg) sudan IV, 100 mg bromcresol green, and 200 mg congo red to 50 milliliters (ml) ethanol. Shake, then dispense in a bottle with a dropper. Do not filter the mixture.

Method

Add 10 ml of chloroform and 10 ml of water to a clean separatory funnel. The two liquids form two immiscible phases. Shake the indicator dye mixture and add three drops of it to the funnel.

Shake the funnel gently once or twice and observe the partition of the solutes in the liquid phases. After settling, collect the lower phase into a test tube or beaker and set it aside. To the separatory funnel, add another 10 ml of chloroform, make another separation, and collect the lower phase into another test tube. Repeat this step once again and compare the collected lower phases from the three consecutive extractions.

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[1]See *Source List.* Small quantities of the materials required to carry out each of the procedures described in this pamphlet may be secured at nominal cost from Russler Glassblowing Services, Post Office Box 3577, Baltimore, Maryland 21214.

[2]If separatory funnels are not available, the procedure may be carried out in a test tube. In this case, the layers are carefully separated by use of an ordinary glass pipet or medicine dropper.

[3]The same indicator-dye mixture is used in Procedures 1 and 2.

The students will note that practically all of the red color of the sudan IV is obtained in the first extraction and very little in the second and third extractions. This reflects the highly soluble nature of this dye in chloroform. In contrast, the indicator dyes bromcresol green and congo red, being more water soluble, are left behind in the water phase. Because of widely differing partition coefficients, therefore, this mixture is easily resolvable in only a few extractions.

Part B: Separation of Solutes by Countercurrent Distribution

When solutes are not clearly differently soluble in two immiscible solvents (for example, when they have very similar partition coefficients or when complex mixtures of solutes are to be separated), a greater number of separatory funnels is required to effect a separation. Because separatory funnels are expensive and tend to become cumbersome when used in larger quantities, it becomes more practical to use a series of test tubes. Such a series makes more separatory steps possible; hence, a more efficient separation of components. This technique is known as countercurrent distribution.

Materials

5 test tubes and rack
Pipet and rubber bulb
Chloroform
Indicator dye mixture[3]

Method

To the first of the 5 test tubes placed in a rack, add 2 ml of chloroform and 2 ml of water. To each of the remaining 4 tubes add only 2 ml of chloroform (This volume can be approximate). To the first tube, add 1 drop of the indicator dye mixture and mix by gentle shaking. Allow to settle, then transfer the upper water layer to the second tube with a pipet and rubber bulb and again mix. Continue this procedure by successively mixing each tube and transferring the upper water layer to each of the remaining tubes. When the water layer reaches the last tube, add 2 ml of water to tubes 1 through 4 only. Mix the layers in each tube, allow to settle, then compare the colors of both phases of all tubes against a background of white paper.

The students will observe that this procedure has resulted in a good separation of the chloroform-soluble sudan IV, which appears to remain in the first tube in contrast to the water-soluble bromcresol green and congo red dyes which appear to move into the last tube. The intermediate tubes 2, 3, and 4 contain very little of each of the components in the respective phases.

It will be easy for the students to comprehend that for complex mixtures or for mixtures containing very closely related solutes, a large number of test tubes might be required to effect a reasonable separation. Some countercurrent distribution systems, for example, are composed of several hundred test tubes.

These procedures serve to illustrate effectively that it is possible to separate a mixture of solutes by taking advantage of the solubility characteristics of the components in two immiscible solvents. The second procedure (Part B) allows a more convenient and more efficient separation than does the first procedure (Part A).

PROCEDURE 2: SEPARATION OF SOLUTES BY PAPER PARTITION CHROMATOGRAPHY

A refined application of the separatory funnels or test tube procedures can be shown by paper chromatography. Paper chromatography employs filter paper as a solid supporting medium, but utilizes the same principles as those involved in solute separation in a separatory funnel or test tubes. The paper, because of the porous nature of the cellulose matrix, can be considered equivalent to a series of test tubes involving an infinitely large number of separatory steps. In a closed vessel containing a solvent of chloroform and water, an equilibrium is reached when the chloroform becomes saturated with water and the water becomes saturated with chloroform. This occurs in both the liquid and the vapor phases. If a solute mixture is placed as a spot on a strip of paper which is dipped into the solvent, a solvent front appears to move across the spot to bring about a separation of the components as the solvent advances. Since the cellulose matrix of the filter paper has such a high affinity for water, it binds it firmly. Thus, the water phase appears to remain stationary and constitutes the non-mobile *stationary phase* of the chromatographic system; whereas the chloroform, being immiscible with water, appears to move freely as the *mobile phase* of the system and the immiscibility of the two solvents is enhanced. Such a two-phase system can be efficiently utilized for the separation of the components according to their partition coefficients.

Adsorption of the solutes on the cellulose support may also occur. Separation is possible in this case not only because of solubility of solutes in two immiscible solvents, but also because of the structural features of the solute molecules which allow for differential attraction to the stationary support.

In paper chromatography, an important term used to denote the distance of movement of solutes is

designated as the *R_f value.* R_f is an expression of a ratio equal to the distance a substance moves with reference (R) to the front (f). The *front* designates the actual solvent line formed as the solvent advances up or down the paper by capillary action. If the solvent moves upward, the technique is referred to as *ascending chromatography;* if it moves downward, *descending chromatography* is observed. To understand the R_f value one can consider the situation in which a solute moves 6 centimeters (cm) on paper when the solvent front has moved 10 cm. The ratio is then 0.6; if the solvent front advances only 9 cm and the solute moves 6 cm, then the ratio becomes 6/9 or 0.666. The R_f value makes possible the qualitative description of the comparative movement of compounds in chromatography.

Materials

Indicator dye mixture[3]
Test Tube[4] – 200 x 25 millimeters (mm)
Filter paper strip (Whatman No. 4,
2 x 24 centimeters (cm)
Cork stopper – No. 10
Wood applicator sticks
Chloroform

Part A: Separation Based on Solvent Partition

Method

On the strip of filter paper, draw a pencil line 3 cm from one end and place at the midpoint of this line a very small drop of the indicator dye mixture (shake the mixture before use) with an applicator stick. The diameter of the spot should be about 0.5 cm or less. After spotting the paper, prepare the test tube "chamber" for chromatography by adding 2 ml of chloroform to a completely dry[5] test tube[6]. Gently lower the filter paper into the test tube, and with the cork stopper, loosely placed in the mouth of the test tube, adjust the position of the strip so that the spotted end of the paper is just immersed in the chloroform. The applied spot should *not* be touching the upper surface of the chloroform. Carefully seat the cork stopper into the test tube without buckling the paper.

As the organic solvent passes over the applied spot, a separation of the components occurs. As in Procedure 1, the chloroform-soluble sudan IV begins to separate almost immediately, contrasted with the water-soluble components which appear to remain at the starting line. The chromatogram should be removed when the solvent front has approached the uppermost third of the strip.

Remove the filter paper strip, mark the position of the solvent front, and dry the paper strip for three or four minutes. The students should be able to explain this separation on the basis of partitioning and solubility.

Part B: Separation Based Upon Adsorption

Using a stationary support such as filter paper to effect resolution, solute adsorption may be involved in the chromatographic process. In Part A of this procedure, separation occurred primarily by a partitioning of the solutes in the two immiscible solvents. The filter paper provided the stationary support for the separation. It is also possible to separate the dyes which remained at the starting line in Part A, by taking advantage of an adsorptive affinity of the indicator dyes for the cellulose base of the paper. In this case, the dyes are solubilized in *one* solvent, water, and separation occurs primarily by a differential adsorption of the various components on the filter paper.

Method

After drying the chromatogram used in Part A, place the filter paper strip in 2 ml water in another clean, dry test tube "chamber." Separation begins as soon as the water flows over the dye mixture. Allow the separation to proceed until the fastest-moving component approaches a point midway between the slowest-moving component and the previously separated chloroform-soluble component.

The components have separated by selective adsorption of the indicator dyes on the filter paper. One of the components is more soluble in water than the other; hence, it moves farther.

PROCEDURE 3: SEPARATION OF SUGARS BY UNIDIMENSIONAL ASCENDING PAPER CHROMATOGRAPHY

In the previous procedures, the indicator dyes were easily separable because they had widely differing partition coefficients, and were directly observable because they were colored. This procedure extends the earlier observations by demonstrating that

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[4]Recommended size. Other sizes may be used. In this case, cut filter paper accordingly. Do not allow paper to touch sides of test tube. The final strip should not buckle and should be long enough to protrude above the cork stopper.

[5]Water is not added to the system since the cellulose in the filter paper attains enough water vapor from air to insure the adequacy of the water phase in this system.

[6]This procedure involving the use of a test tube and cork is similar to one described by Rockland and Dunn [2].

paper chromatography can also be utilized for the more difficult separation of closely related, non-colored compounds which can be visualized by appropriate dipping techniques. In this case, three sugars are separated in ordinary canning jars by unidimensional ascending paper chromatography.

Materials

2-quart wide-mouth Mason (canning) jar with cover (or other appropriate screw-cap wide-mouth jar)
Aluminum foil (12 or 13 cm square)
Filter paper – Whatman No. 4 (20 cm square)
Wood applicator sticks
Stapler mounted on bench with 2 "C" clamps (see Figure 1)

Aqueous sugar solutions:
(1) 1 percent glucose
(2) 2 percent ribose
(3) 3 percent lactose
(4) a mixture containing
1 percent glucose
1 percent ribose
3 percent lactose

Solvent:
ethyl acetate . . . 12 parts
pyridine . . . . . 5 parts
water . . . . . 4 parts

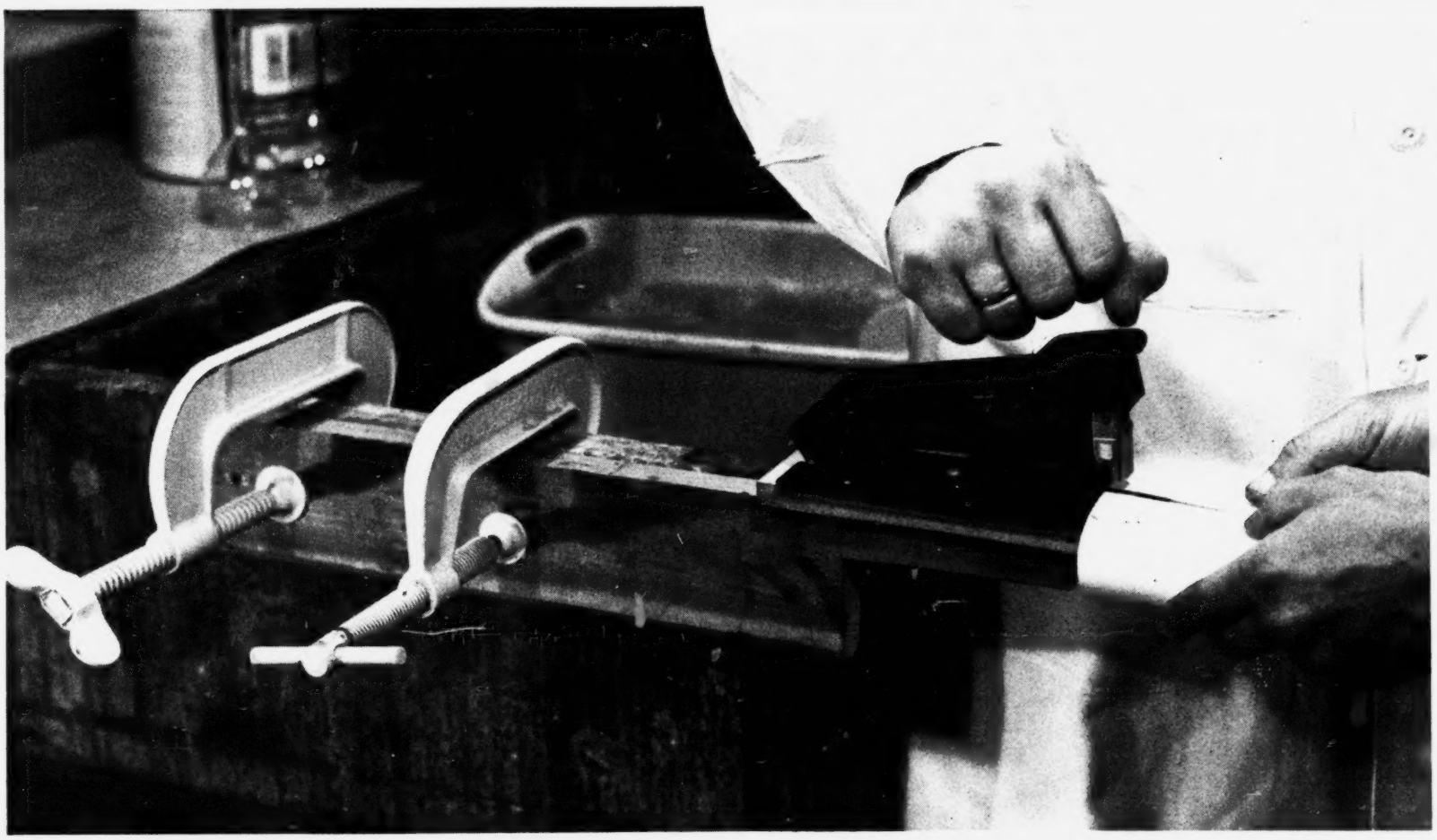
Dip or spray reagents for sugar localization:
(1) silver nitrate
(saturated solution in water). . . . . 0.1 volume
(2) acetone . . . . . 20 volumes
(3) 0.5 percent sodium hydroxide (NaOH) in ethanol
(4) 2N ammonium hydroxide (NH_4OH) if required

Method

Prepare the wide-mouth jar for use as a chamber by adding to the jar 60 ml of a mixture of the solvent ethyl acetate-pyridine-water. To prevent possible solvent attack upon the cover, place the piece of aluminum foil over the top of the jar, and replace the cover of the jar securely. Allow the solvents in the chamber to reach equilibrium. (Avoid unnecessary exposure to the solvent mixtures since pyridine has an unpleasant odor.)

Figure 1. Device for forming paper cylinders.

Nail a block of wood perpendicularly to the tapered end of a wooden support. Mount a stapler on the block of wood, and clamp the support to a bench. To use the stapler, one person holds the paper and another staples. The edges of the paper should not quite touch.



After the student's name is written on the filter paper, draw a starting line *in pencil* across one dimension of the paper, 3 cm from the edge. Place four *pencil* dots along the line at 3-cm intervals. Label 3 dots with the names of the sugars and the fourth dot "mixture." The sugar samples are to be placed upon the dots in such a manner as to prevent their spread beyond a 0.5-cm diameter circle. Large spots will interfere with separation.

Immerse a separate applicator stick in each of the sugar solutions to be analyzed, and touch each stick perpendicularly to the appropriate starting line dot. (If desired, a sugar "unknown" may also be applied to a starting line dot.) After the spots are dry, staple the paper without overlapping, along the length of the paper at both ends and in the center as shown in Figure 1.

Place the paper cylinder carefully into the jar, replace the jar cover, and "develop" the chromatogram for 1½ hours or longer, if time permits. However, the chromatogram should be removed before the solvent front reaches the upper edge of the cylinder so that R_f values can be calculated.

After development, remove the chromatogram from the chamber by catching a small hook (a bent paper clip will do) on the top staple and mark the position of the solvent front in pencil. Dry it in a hood or other well-ventilated area.

Remove the staples from the cylinder and dip the chromatogram rapidly through a saturated solution of the silver nitrate in acetone, to localize the sugars on the dried chromatogram (3). The precipitate formed in the reagent does not interfere with the procedure. Avoid actual contact with the silver nitrate reagent to prevent discoloration of hands. Allow the excess acetone on the paper to drain, then evaporate. When it is dry, dip the paper through the sodium hydroxide in another tray and again allow it to drain and dry. Excessive background discoloration may be neutralized by passing the chromatogram through another solution of 2N ammonium hydroxide. The spots appear immediately.

To calculate the R_f value of each sugar, record the distance from the center of each spot to the starting line and the distance which the solvent front traveled. A comparison of the R_f values of the "known" and "unknown," if any, should enable the students to identify the sugars.

Both solvent partition and adsorption on the filter paper played a role in this separation. Since all of these sugars are very water-soluble, the adsorption was more significant. This can be explained on the basis of the molecular structure of the sugars. Ribose is a 5-carbon pentose and moved fastest, glucose is a 6-carbon hexose and moved to an intermediate position, and lactose is a 12-carbon disaccharide and moved slowest in this system. Each carbon atom of the sugar has a hydroxyl group attached. By hydrogen bonding these hydroxyl groups have a high affinity for the many hydroxyl groups contained in the cellulose of the filter paper matrix. In general, the more solute hydroxyl groups there are on an atom, the more attraction it has for the filter paper support, and the slower moving the solute in this chromatographic system.

PROCEDURE 4: TWO-DIMENSIONAL PAPER CHROMATOGRAPHY OF AMINO ACIDS

The separation of the sugars in a single ascending dimension was possible because the partition coefficients were differentiated enough in solubility and adsorptive characteristics. In contrast, when the components of a complex mixture have very similar partition coefficients, as in the case of amino acids, some overlapping of the positions taken by the amino acids would occur in the unidimensional paper chromatogram and differences would not be distinguishable. It would be possible, however, to obtain good separation if, after separating the components in one dimension, one dried the paper, turned it 90 degrees, and introduced it into a different solvent so that the solvent front moves at a right angle to the original direction. The second solvent is such that the partition coefficients of those components which overlapped are now sufficiently different to permit better separation. This process is called two-dimensional paper chromatography.

Materials

Two 2-quart wide-mouth Mason (canning) jars with covers (or other appropriate screw-cap, wide-mouth jars)
Aluminum foil (12 or 13 cm square)
Filter paper – Whatman No. 4 (20 cm square)
Wood applicator sticks
Stapler mounted on bench with 2 "C" clamps (see Figure 1)
Solvents: Prepared by volume

First dimension (Solvent 1)

Chloroform	40 parts
Methanol	40 parts
Ammonium hydroxide (concentrated)	20 parts

Second dimension (solvent 2)

Butanol	60 parts
Glacial acetic acid	20 parts
Water	20 parts

Amino acid mixture
- arginine
- lysine
- glutamic acid
- glycine
- alanine
- valine
- leucine
- phenylalanine
- tyrosine
- histidine

The mixture is composed of a 0.1 percent aqueous solution of each amino acid in 10 percent isopropanol (noncolored alcohol) and 2 percent hydrochloric acid (6M).

Reagent for localization
0.25 percent ninhydrin in acetone (do not place in tray until ready for use).

METHOD

Add 50 ml of Solvent 1 to one jar and 50 ml of Solvent 2 to the second jar, then cover the tops with aluminum foil, and cap the jars. Next, draw a 0.5-cm circle at a point 2 cm from each edge of the lower right hand corner of the filter paper. Within this circle, apply some of the amino acid mixture with a wooden applicator stick so that the solution fills the circle. Immediately after application allow the spot to air dry.

Roll the filter paper into a cylinder and carefully staple the edges at the top, middle, and bottom with a stapler, as shown in Figure 1. The edges should approximate each other, but should not be touching. (Avoid handling the paper excessively so that the free amino acids found in skin secretions do not cause extraneous spots to appear on the finished chromatogram.)

Remove the cover of the jar containing Solvent 1, and lower the paper cylinder into the jar[7] so that the amino acid spot is near, but not touching, the solvent surface. Replace the aluminum foil and the lid. Without disturbing the jar, allow approximately 40 to 50 minutes for the solvent to ascend the paper until the front is approximately 2 cm from the top. Remove the paper with a bent paper clip and dry in a hood.

After drying the paper, carefully remove the staples and form a cylinder in the second dimension. Staple the paper, place it in the jar[7] containing solvent 2, and allow for development in this dimension (approximately 2 to 2½ hours). Remove the paper and dry in a hood or other well-ventilated area.

Remove the staples from the dried paper, hold it diagonally corner to corner and pass it rapidly, face up, through a solution of ninhydrin reagent which has been added to a tray just before use. Allow the paper to drain, then transfer the paper to an oven (75 degrees C) for three minutes, or allow it to dry in the air for slower color development. The spots should be outlined with pencil and the color of each noted immediately since the colors will fade with time.

Compare the separated amino acid positions with those given in the standard map (Figure 2). Note also the variety of colors given by the amino acids.

The similar structural characteristics of the amino acids require a two-dimensional chromatographic system to assure adequate separation. A disadvantage of this method is the longer development times required in the two systems. Faster systems have been reported, but these are associated with poorer resolution. However, the method is valuable in that a wide variety of food proteins may be analyzed after hydrolysis into their constituent amino acids. By using a variety of solvent systems, one can determine the amino acid content of a large number of proteins.[3]

PROCEDURE 5: SEPARATION OF DYES BY THIN-LAYER CHROMATOGRAPHY

An elegant refinement of chromatography, introduced within the last few years, involves the separation of substances on a thin layer of adsorbent materials; hence the name *thin-layer chromatography*. The procedure is similar to that of paper chromatography in that principles of solvent equilibrium, solvent partition of solutes, and adsorption are involved. It differs from paper chromatography in that the solid adsorbent permits a greater quantity of material to be applied and the solid supporting medium, usually glass or film, is not involved in the separatory process which includes principles of elution analysis and adsorption isotherms. [4]

In thin-layer chromatography, an *adsorbent medium,* commonly silica gel and a binder, is used to coat a solid support such as glass or plastic. The material to be analyzed is spotted upon the adsorbent material, and the latter is developed in a solvent system in a closed atmosphere to allow separation of the components. The greatest advantages of thin-layer chromatography are its speed, greater

[7]Each dimension of the paper should be developed in the direction indicated on the amino acid map shown in Figure 2.

sensitivity, and resolving power. Consequently, the technique is much more efficient than is paper chromatography. All of the separations described in Procedures 1 through 4 can be accomplished with thin-layer chromatography techniques, but in some cases, different solvent systems would need to be employed. The necessary modifications are described in detail in the literature. [4,5]

To demonstrate the effectiveness of the thin-layer technique, a mixture of three dyes is separated in a very simple system using a thin layer of silica gel on film.

Materials

Test tube - 25 x 200 mm
Cork stopper - No. 10
Thin-layer chromatography strips cut to fit test tube
Dye mixture for thin-layer chromatography[8]
Wood applicator sticks
Chloroform

Method

To the dry test tube, add 1 ml of chloroform and cover with the cork stopper to assure equilibrium.

With an applicator stick, apply a very small spot of the dye mixture 1 cm from one end of the thin layer strip. Remove the cork from the test tube and introduce the strip carefully into the test tube, which is held at an angle. Return the tube to a vertical position and recap with the stopper. Observe the very rapid separation of the components of the dye mixture which should separate in ten to fifteen minutes. Remove the strip after the separation is complete. Calculate the R_f values.

Upon completing this procedure, the students will be impressed with the simplicity and tremendous separatory power of this technique. The speed of separation and a comparison of the results of thin-layer and paper chromatography will make clear the advantages of the former over the latter technique.

SOURCE LIST

Small quantities of materials used in the procedures may be procured from the dealer listed in footnote 1.

Procedures I and II:

Indicator dye mixture: SX1115 Sudan IV, BX1160 Bromcresol green, and CX1905 Congo red are available from Matheson Coleman & Bell, Post Office Box 85, East Rutherford, New Jersey 07073.

Procedures II, III, IV:

Filter paper (Whatman No. 4) is available from H. Reeve Angel and Co., Inc., Clifton, New Jersey 07014.

Wood applicator sticks are available from Peerless Wood Applicators, Diamond Match Company, B.F.D. Divisions, 733 Third Ave., New York, New York 10001.

Procedure IV:

The amino acid mixture is included in Kit No. 20 available from Nutritional Biochemicals, 21010 Miles Avenue, Cleveland, Ohio 44128.

The amino acid reagent, 1X45 Ninhydrin, is available from Matheson Coleman & Bell, Post Office Box 85, East Rutherford, New Jersey 07073.

The reagent can also be purchased as an aerosol spray called Spray-Tec No. 101 Ninhydrin in Acetone, available from Mann Research Laboratories, 136 Liberty Street, New York, New York 10006.

Procedure V:

The thin-layer sheets are Eastman Chromagram Sheets, Type K 301R2, and are available from Distillation Products Industries, Rochester, New York 14603.

The dye mixture is Eastman Test Dye Mixture for TLC No. 6077, available from Distillation Products Industries, Rochester, New York 14603.

REFERENCES

1. Heftmann, E. *Chromatography.* Reinhold Publishing Corp., New York, 1961.

2. Rockland, L. B., and Dunn, M.S. "Capillary-Ascent Test Tube Method for Separating Amino Acids by Filter Paper Chromatography." *Science* 109: 539-40; May 27, 1949.

3. Smith, I., Editor. *Chromatographic Techniques.* William Heinemann, Ltd., London, England. 1958.

4. Stahl, Egon, Editor. *Thin-Layer Chromatography: A Laboratory Handbook.* Academic Press, Inc., New York. 1965.

5. Randerath, Kurt (translated by D.D. Libman). *Thin-Layer Chromatography.* Academic Press, Inc., New York, 1963.

[8] Do not use the indicator-dye mixture used in Procedures 1 and 2. See footnote 1 or *Source List* for procuring the dye mixture used in Procedure 5.

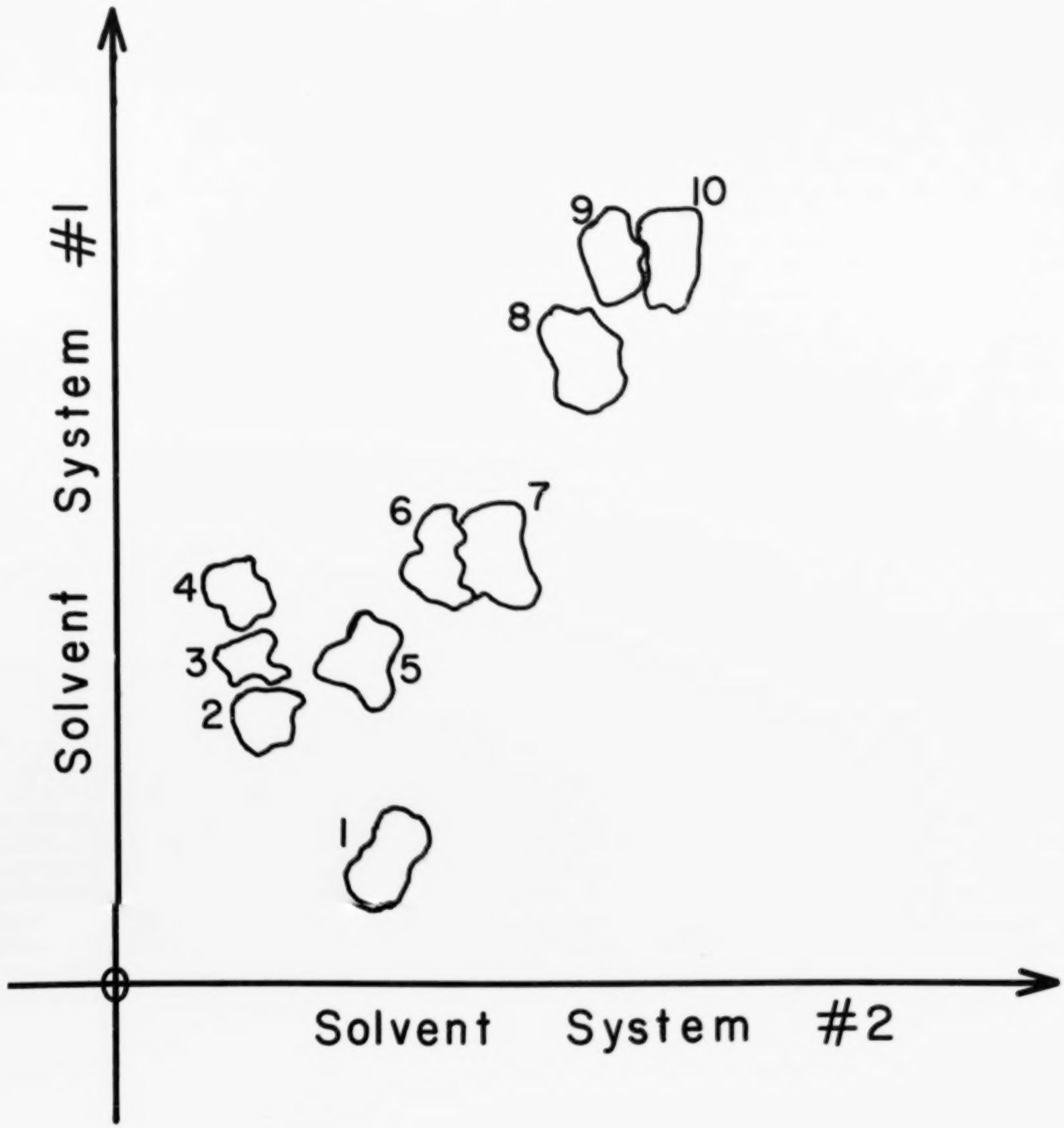


Figure 2.

Map of amino acid mixture separated by two-dimensional paper chromatography. Origin is at intersection of ordinate and abscissa.

1. *Glutamic acid*
2. *Arginine*
3. *Histidine*
4. *Lysine*
5. *Glycine*
6. *Alanine*
7. *Tyrosine*
8. *Valine*
9. *Phenylalanine*
10. *Leucine*

NOTES

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